1242-Pos Board B12
Chloride Flux in Prestin-Expressing Cells
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In prestin-expressing cells, intracellular Cl^- ($[Cl^-]_i$) flux plays a preeminent role in promoting prestin activity, especially since the resulting V_h shift in prestin's state-probability function (inferred from nonlinear capacitance - NLC) along the V_m axis will effect a motile response. For example, with perforated patch clamp and local perfusion, changing extracellular Cl concentration from 1 mM to 140 mM using prestin's NLC as a measure of intracellular Cl indicates a several mM increase in intracellular Cl concentration. Nevertheless, the mechanism underlying Cl^- flux in prestin-expressing cells is not clear.
To better define Cl^- flux, the CFP-YFP-based ratiometric Cl indicator (Cl-sensor, J. Neurosci. Meth., 2008, 170, 67) was modified to reduce pH sensitivity by shifting the *pKa* away from the physiological pH range. Using excitation ratiometric imaging of fluorescence, $[Cl]_i$ was measured in either induced- or non-induced prestin-expressing HEK cell-lines transfected with our new Cl-sensor construct. Upon changing local extracellular perfusion from 0.2 mM to 140 mM Cl buffer, fluorescence measures indicate a significant difference between induced- and non-induced prestin cell-lines.
We also evaluated $[Cl]_i$ flux using a Cl-sensor fusion product of prestin at the C-terminal to better gauge flux near prestin's intracellular binding site. Comparison of $[Cl]_i$ flux in HEK-293T cells transfected with a normal prestin-Cl-sensor or a prestin construct with a C-terminal deletion that eliminated NLC (P709-Cl-sensor) showed no statistical difference in flux. These data suggest that prestin's NLC is separable from Cl^- movement induced by prestin, as we previously suggested (Bai et al., BJ, 3179, 2009). Further experiments are underway, including voltage dependence of flux by simultaneously monitoring fluorescence ratio signals and NLC using perforated patch.
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1243-Pos Board B13
Charge Induced Activation of MscL: Probing the Hydrophobic Gate Region
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Mechanosensitive Channel of Large Conductance (MscL) are non selective channels that serve as an emergency valve for bacterial osmoregulation.Studies (Yoshimura et al 1999 and 2001;Blount et al 1996)done in *E.coli* have shown that MscL function in response to the change in membrane tension as well as by introducing charge at the pore. Here we showed that charge-induced activation of MscL from *Lactococcus lactis* (LlMscL) and *Mycobacterium tuberculosis* (TbMscL) resemble that of the widely studied MscL from *E.coli*. Cysteine mutants around the hydrophobic gate region of these channels were created and used for channel activation both *in vivo* and *in vitro* using a charged chemical compound, MTSET. TbMscL cysteine mutants showed a different charge-sensitivity pattern compared to LlMscL mutants. While only 20 and 21st amino acid positions of TbMscL were responsive to MTSET, 20, 21, 22 and 23rd positions were responsive in the case of LlMscL. These results suggest that it is possible to activate MscL from other organisms by charging their hydrophobic pore region. Furthermore, although MscL from different organisms show highest sequence identity around the pore, individual MscL retain their own characteristics.

1244-Pos Board B14
The Use of Engineered Voltage-Sensing Phosphatases to Study the Determinants of Substrate Specificity of Phosphoinositide Phosphatases
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Ciona intestinalis voltage sensing phosphatase (Ci-VSP) is the founding member of a new class of voltage-sensing proteins, and exhibits $PI(3,4,5)P_3$ and $PI(4,5)P_2$ 5'phosphatase activity upon membrane depolarisation (Murata and Okamura, 2007; Iwasaki et al., 2008; Halaszovich et al., 2009). Ci-VSP consists of a N-terminal voltage sensing domain (VSD) and a C-terminal phosphatase domain (PD) that is highly homologous to the phosphoinositide (PI) phosphatase PTEN (Murata et al., 2005). Recently, we reported on a voltage-sensitive chimera of the Ci-VSP voltage-sensor and the tumour suppressor PTEN (Ci-VSPTEN) in agreement with PTEN's 3'phosphatase activity (Lacroix et al., 2011). Here we extend this knowledge to a novel chimera of the VSD of Ci-VSP and the PTEN homologue TPIPα (also known as TPTE2), termed Ci-VSP/TPIP (Walker et al., 2001).
Using whole cell voltage-clamp and total internal reflection microscopy with genetically-encoded phosphoinositide sensors, we show that Ci-VSP/TPIP is a voltage-sensitive $PI(3,4,5)P_3$ and $PI(4,5)P_2$ 5'phosphatase in contrast to previous reports of TPIPα as a 3'phosphatase (Walker et al., 2001). The voltage dependence of Ci-VSP/TPIP was shifted to hyperpolarized potentials compared to Ci-VSP. Functional comparisons of Ci-VSP, Ci-VSPTEN and Ci-VSP/TPIP identified alanin/glycine 365 as the critical determinant of PTEN's substrate specificity, since Ci-VSPTEN(A365G) produced robust voltage-dependent $PI(3,4,5)P_3$ 5' phosphatase activity, in contrast to the 3'phosphatase activity of wild-type PTEN.
In conclusions, our data characterise the 5'phosphatase Ci-VSP/TPIP and reveal that engineered voltage-sensing phosphatases can be used to study the activity and substrate specificity of the important tumour suppressor PTEN *in vivo*.
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1245-Pos Board B15
A Novel Rotation Mechanism of F1-ATPase Based on the Water-Entropy Effect
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We propose a novel picture of the rotation mechanism of F_1-ATPase [1]. In the proposal, the key factor is the translational entropy of water, which has been shown to drive a variety of self-assembly processes in biological systems [2]. We calculate the hydration entropies of three different sub-complexes comprising the gamma subunit, one of the beta subunits, and two alpha subunits adjacent to them. The calculation is made using the angle-dependent integral equation theory combined with the multipolar water model [3] and morphometric approach [4]. The major finding is that the packing in F_1-ATPase is highly asymmetrical and this asymmetry is ascribed to the water-entropy effect. We discuss how the rotation of the gamma subunit is induced by such chemical processes as ATP binding, ATP hydrolysis, and release of the products. In our picture, the asymmetrical packing plays crucially important roles and the rotation is driven by the water-entropy effect. As part of the demonstration of the validity of our rotation mechanism, we also analyze the water-entropy change in yeast F_1-ATPase during 16-degree rotation of the gamma subunit [5]. The result demonstrates the validity of the water-entropy mechanism proposed in Ref 1.
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1246-Pos Board B16
Rotary Motion of F_OF_1-ATP Synthase in the Presence of *pmf*
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F_oF_1-ATP synthase (F_oF_1) is a molecular motor protein, which synthesizes ATP coupling with clockwise rotations driven by the proton motive force (*pmf*) and also reversibly hydrolyzes ATP which drives anticlockwise rotations to form the electrochemical proton gradient. Extensive studies revealed the rotary catalytic mechanism of ATP-driven rotation of F_oF_1; however, that of *pmf*-driven rotation was poorly understood. Therefore, we tried to characterize the *pmf*-driven rotation of F_oF_1 by controlling the amplitudes of *pmf*. In this study, we reconstituted F_oF_1 into the supported membrane formed on the Ni-NTA modified coverglass and visualized the rotary motion by attaching the magnetic beads (ϕ=~200nm) on the rotor part. Furthermore, we developed the novel experimental setup which enabled us to modulate the amplitudes of *pmf*. Based on these experimental setups, we obtained a few primitive data of rotary motion sensitive to *pmf* as follows. When we generated an electric potential difference ($\Delta\Psi$) against ATP-driven rotating F_oF_1, F_oF_1 showed one reverse rotation in clockwise as expected. In addition, after redusing $\Delta\Psi$, F_oF_1 restarted to rotate in anticlockwise. Currently we are collecting more experimental data for the